

In the Claims

This listing of claims will replace all prior versions and listings of claims in this application.

1 (currently amended). A method for producing a heterodimeric specific wild type- or chimeric T-cell receptor (TCR) in a manner such that externally provided TCR-chains do pair and do not form mixed pairs with endogenous TCR-chains of T-cells, and wherein TCR functionality and stability is not impaired, wherein the heterodimeric TCR comprises containing a first chain and a second chain that interact one with another at the at least one surface of their amino acids, wherein the at least one amino acid-surface is subjected to a rational mutagenesis, such that the at least one amino acid-surface of the first chain or the amino acid-surface of the second chain comprises a sterically projecting group, which interacts with a sterically recessed group on the at least one amino acid-surface of the corresponding first chain or second chain, said method comprising the steps of:

(a) providing DNA-molecules, comprising the coding regions for the at least one amino acid-surface to be mutated of the first chain or second chain, in (a) joint or separate mutagenesis-vector system(s),

(b) mutagenesis of the DNA-molecules, wherein the nucleic acid sequence encoding [[for]] the at least one amino acid-surface is modified compared to the initial sequence in such a manner that in the at least one amino acid-surface of the first chain or the at least one amino acid-surface of the second chain, a sterically projecting group is introduced, and in the corresponding at least one interacting amino acid-surface of the second chain or the first chain, a sterically recessed group is introduced, compared to the initial amino acid-surface(s), whereby individual mutated fragments are produced,

wherein the amino acid as introduced after the mutagenesis of the DNA-molecules that introduces the sterically recessed group compared with the initial amino acid-surface is glycine, serine, threonine, valine, or alanine, and wherein the amino acid as introduced after the mutagenesis of the DNA-molecules that introduces the sterically projecting group compared with the initial amino acid-surface is selected from glutamine, glutamic acid, alpha-methylvaline, histidine,

hydroxylysine, tryptophan, lysine, arginine, phenylalanine, or tyrosine, and

c) translation of at least two of the single mutated fragments from step b), such that the pairing of the heterodimeric specific first-chain/second-chain TCR being mutated at least one amino acid-surface is selectively promoted, and

d) presentation of the heterodimeric first-chain/second-chain TCR by a T-cell.

2 (previously presented). The method according to claim 1, wherein step c) is replaced by the following steps:

(c') optionally, sub-cloning of the mutated fragments into a suitable transfection-vector system,

(c'') transfection or co-transfection or transduction of at least two of the mutated fragments into a mutant TCR-deficient T-cell, and

(c''') expression of the heterodimeric first-chain/second-chain TCR in a recombinant T-cell.

3 (currently amended). The method according to claim 1, wherein step c) is replaced by the following steps:

c') *In vitro*-translation or *in vivo*-translation of at least two of the individual mutant-fragments from step b) and, optionally, subsequent isolation and/or purification of the translated mutant-fragments,

such that the pairing of the heterodimeric specific first-chain/second-chain TCR being mutated at least on one amino acid-surface is selectively promoted, and

c'') introduction of the mutated specific first-chain/second-chain TCR into a T-cell.

4 (previously presented). The method according to claim 3, wherein the *in vivo* translation takes place in a host cell.

5 (previously presented). The method according to claim 3, wherein the introduction takes place by liposome-transfer.

6 (currently amended). The method according to claim 1, wherein the TCR is an alpha/beta TCR, a gamma/delta TCR, a humanized or partially humanized TCR, a TCR being provided with additional (functional) domains, or a TCR being provided with alternative domains.

7 (previously presented). The method according to claim 1, wherein the amino acids as introduced after the mutagenesis of the DNA-molecules are further suitably chemically modified, in order to thereby introduce a sterically projecting group or a sterically recessing group.

8 (previously presented). The method according to claim 1, wherein the amino acids as introduced after the mutagenesis of the DNA-molecules directly provide the sterically projecting group or the sterically recessing group.

9 (previously presented). The method according to claim 1, wherein the amino acids as introduced by the mutagenesis of the DNA-molecules are chosen in such a manner that a mutual exchange of the amino acids on the surfaces of the interacting chains of the TCR is achieved.

10-11 (cancelled).

12 (previously presented). The method according to claim 1, wherein at least two surfaces of a TCR-chain are simultaneously subjected to mutagenesis.

13 (previously presented). The method according to claim 1, wherein the corresponding interacting surfaces are localized in the variable domains of the TCR-chains.

14 (previously presented). The method according to claim 1, wherein the corresponding interacting surfaces are localized in the constant domains of the TCR-chains.

15 (previously presented). The method according to claim 1, wherein the domains of the TCR-chains to be mutated are selected from mammalian domains.

16 (previously presented). The method according to claim 1, wherein the rational mutagenesis of the TCR-chains at the same time leads to a humanization of the TCR.

17 (previously presented). The method according to claim 1, wherein the alpha- and beta-chains of an MDM2(81-88)-specific TCR are used as alpha-chain and beta-chain, and wherein the Gly192 of the constant region of the alpha-chain and the Arg208 of the constant region of the beta-chain are exchanged by Arg 192 in the constant region of the alpha-chain and by Gly208 in the constant region of the beta-chain.

18 (previously presented). The method according to claim 17, wherein simultaneously with or subsequent to the exchanges at positions 192 and 208, additional positions are modified in the chains.

19 (previously presented). The method according to claim 1, wherein a retroviral vector is used as a transfection system.

20 (withdrawn). A mutated alpha- or beta-chain of a TCR, produced according to a method for producing a heterodimeric specific wild type- or chimeric T-cell receptor (TCR), containing a first chain and a second chain that interact one with another at at least one surface, wherein the at least one surface is subjected to a rational mutagenesis, such that the at least one surface of the first chain or the surface of the second chain comprises a sterically projecting group, which interacts with a sterically recessed group on the at least one surface of the corresponding first chain or second chain, comprising the steps of:

- (a) providing DNA-molecules, comprising the coding regions for the at least one surface to be mutated of the first chain or second chain, in (a) joint or separate mutagenesis-vector system(s),

(b) mutagenesis of the DNA-molecules in a manner known as such, wherein the nucleic acid sequence encoding for the at least one surface is modified compared to the initial sequence in such a manner that in the at least one surface of the first chain or the at least one surface of the second chain, a sterically projecting group is introduced, and in the corresponding at least one interacting surface of the second chain or the first chain, a sterically recessed group is introduced, whereby individual mutated fragments are produced,

c) translation of at least two of the single mutated fragments from step b), such that the pairing of the heterodimeric specific first-chain/second-chain TCR being mutated at least one surface is selectively promoted, and

d) presentation of the heterodimeric first-chain/second-chain TCR by a T-cell.

21 (withdrawn). The mutated TCR, according to claim 20, which is mutated MDM2(81-88)-specific TCR (Seq ID No. 2 and Seq ID No. 5).

22 (withdrawn). An isolated nucleic acid, comprising a sequence coding for a mutated alpha- or beta-chain of a TCR produced according to a method for producing a heterodimeric specific wild type- or chimeric T-cell receptor (TCR), containing a first chain and a second chain that interact one with another at at least one surface, wherein the at least one surface is subjected to a rational mutagenesis, such that the at least one surface of the first chain or the surface of the second chain comprises a sterically projecting group, which interacts with a sterically recessed group on the at least one surface of the corresponding first chain or second chain, comprising the steps of:

(a) providing DNA-molecules, comprising the coding regions for the at least one surface to be mutated of the first chain or second chain, in (a) joint or separate mutagenesis-vector system(s),

(b) mutagenesis of the DNA-molecules in a manner known as such, wherein the nucleic acid sequence encoding for the at least one surface is modified compared to the initial sequence in such a manner that in the at least one surface of the first chain or the at least one surface of the second chain, a sterically projecting group is introduced, and in the corresponding at least one interacting surface of the second chain or the first chain, a sterically recessed group is introduced,

whereby individual mutated fragments are produced,

c) translation of at least two of the single mutated fragments from step b), such that the pairing of the heterodimeric specific first-chain/second-chain TCR being mutated at least one surface is selectively promoted, and

d) presentation of the heterodimeric first-chain/second-chain TCR by a T-cell.

23 (withdrawn). A composition selected from the group consisting of:

a) a DNA- or RNA-vector molecule, comprising at least one nucleic acid according to claim 22, and that can be expressed in cells;

b) a host cell containing a DNA- or RNA-vector molecule comprising at least one nucleic acid according to claim 22; and

c) a recombinant T-cell, expressing at least one mutated alpha- or beta-chain of a TCR, produced according to a method for producing a heterodimeric specific wild type- or chimeric T-cell receptor (TCR), containing a first chain and a second chain that interact one with another at at least one surface, wherein the at least one surface is subjected to a rational mutagenesis, such that the at least one surface of the first chain or the surface of the second chain comprises a sterically projecting group, which interacts with a sterically recessed group on the at least one surface of the corresponding first chain or second chain, comprising the steps of:

(i) providing DNA-molecules, comprising the coding regions for the at least one surface to be mutated of the first chain or second chain, in (a) joint or separate mutagenesis-vector system(s),

(ii) mutagenesis of the DNA-molecules in a manner known as such, wherein the nucleic acid sequence encoding for the at least one surface is modified compared to the initial sequence in such a manner that in the at least one surface of the first chain or the at least one surface of the second chain, a sterically projecting group is introduced, and in the corresponding at least one interacting surface of the second chain or the first chain, a sterically recessed group is introduced, whereby individual mutated fragments are produced,

(iii) translation of at least two of the single mutated fragments from step b), such that the pairing of the heterodimeric specific first-chain/second-chain TCR being mutated at least one

surface is selectively promoted, and

(iv) presentation of the heterodimeric first-chain/second-chain TCR by a T-cell.

24-26 (Cancelled).

27 (withdrawn). A method for treatment of a cancerous disease, comprising providing to a patient in need thereof at least one of the following.

(a) a mutated alpha- or beta-chain of a TCR, produced according to a method for producing a heterodimeric specific wild type- or chimeric T-cell receptor (TCR), containing a first chain and a second chain that interact one with another at at least one surface, wherein the at least one surface is subjected to a rational mutagenesis, such that the at least one surface of the first chain or the surface of the second chain comprises a sterically projecting group, which interacts with a sterically recessed group on the at least one surface of the corresponding first chain or second chain, comprising the steps of:

(i) providing DNA-molecules, comprising the coding regions for the at least one surface to be mutated of the first chain or second chain, in (a) joint or separate mutagenesis-vector system(s),

(ii) mutagenesis of the DNA-molecules in a manner known as such, wherein the nucleic acid sequence encoding for the at least one surface is modified compared to the initial sequence in such a manner that in the at least one surface of the first chain or the at least one surface of the second chain, a sterically projecting group is introduced, and in the corresponding at least one interacting surface of the second chain or the first chain, a sterically recessed group is introduced, whereby individual mutated fragments are produced,

(iii) translation of at least two of the single mutated fragments from step b), such that the pairing of the heterodimeric specific first-chain/second-chain TCR being mutated at least one surface is selectively promoted, and

(iv) presentation of the heterodimeric first-chain/second-chain TCR by a T-cell; or

(b) a recombinant T-cell, expressing at least one mutated alpha- or beta-chain of a TCR, produced according to a method for producing a heterodimeric specific wild type- or chimeric T-cell

receptor (TCR), containing a first chain and a second chain that interact one with another at least one surface, wherein the at least one surface is subjected to a rational mutagenesis, such that the at least one surface of the first chain or the surface of the second chain comprises a sterically projecting group, which interacts with a sterically recessed group on the at least one surface of the corresponding first chain or second chain, comprising the steps of:

- (i) providing DNA-molecules, comprising the coding regions for the at least one surface to be mutated of the first chain or second chain, in (a) joint or separate mutagenesis-vector system(s),
- (ii) mutagenesis of the DNA-molecules in a manner known as such, wherein the nucleic acid sequence encoding for the at least one surface is modified compared to the initial sequence in such a manner that in the at least one surface of the first chain or the at least one surface of the second chain, a sterically projecting group is introduced, and in the corresponding at least one interacting surface of the second chain or the first chain, a sterically recessed group is introduced, whereby individual mutated fragments are produced, and
- (iii) translation of at least two of the single mutated fragments from step ii), such that the pairing of the heterodimeric specific first-chain/second-chain TCR being mutated at least one surface is selectively promoted; and
- (iv) presentation of the heterodimeric first-chain/second-chain TCR by a T-cell.

28 (withdrawn). The method according to claim 27, wherein a cancerous disease is treated that is in connection with a modified expression of MDM2, p53, Her-2/neu, Ras, tyrosinase, MART, Gp100, MAGE, BAGE, MUC-1, CD45, CD19 or PRDI-BF1.

29 (new). The method according to claim 1, wherein mutagenesis of the DNA molecules is in a TCR ecto-domain.

30 (new). The method according to claim 1, wherein mutagenesis of the DNA molecules is in a TCR constant domain.